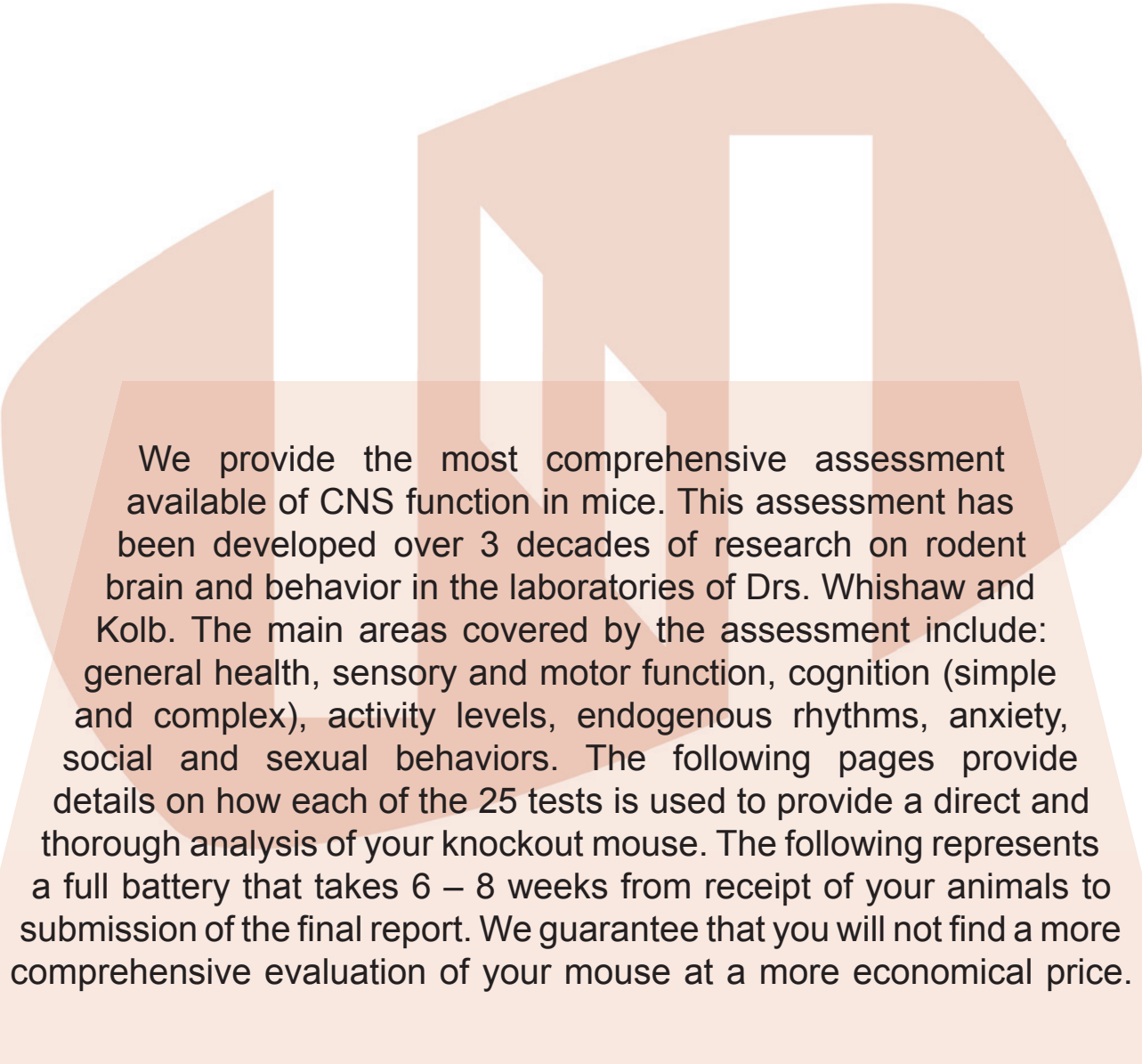


Mouse Phenotyping



We provide the most comprehensive assessment available of CNS function in mice. This assessment has been developed over 3 decades of research on rodent brain and behavior in the laboratories of Drs. Wishaw and Kolb. The main areas covered by the assessment include: general health, sensory and motor function, cognition (simple and complex), activity levels, endogenous rhythms, anxiety, social and sexual behaviors. The following pages provide details on how each of the 25 tests is used to provide a direct and thorough analysis of your knockout mouse. The following represents a full battery that takes 6 – 8 weeks from receipt of your animals to submission of the final report. We guarantee that you will not find a more comprehensive evaluation of your mouse at a more economical price.

NeuroInvestigations

Mouse Phenotyping

Appearance and reflexes

General health
Posture and turning reflex
Sensory orientation
Forelimb strength
Auditory startle
Visual discrimination

Pain perception

Von Frey Hairs
Tail flick
Formalin test

Tests of balance and coordination

The rod test
Rotarod

Tests of cognition

Morris water task
Fear conditioning
Place preference.
Recognition memory
Prepulse inhibition

Tests of activity

Open Field Activity
Circadian Activity

Tests of motor skill

Grid Walk
Reaching task

Tests of anxiety

Fear Potentiated Startle

Species typical behaviour

Social conflict
Sexual behaviour
Nest Building

The procedures for these tests are standardized, and the rationales for use are described in Whishaw et al. (1999).

Appearance and reflexes

General health is assessed in each mouse by noting body weight, body length, eye condition, vibrissae movement, and muscle tone.

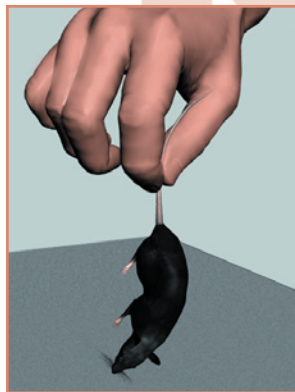
Our **Posture and turning reflex test** has proven to be sensitive to many different kinds of neurological impairments. In this test each mouse is hung repeatedly by the base of the tail over the surface of a table. The direction of body turn, carriage of forelimbs and hindlimbs, and the extension of the digits are scored. A normal mouse will show turns equally often to the right or left, and will carry its body in a species typical manner. When nearing the test table the digits will be extended outward in anticipation of contact with the table.

Sensory orientation involves testing for appropriate orientation to somatosensory, auditory, and visual stimuli.

Forelimb strength is assessed using a grip strength apparatus. Each mouse is made to grasp a handle that is fastened to a calibrated spring. Consistent and increasing pressure is applied by pulling the mouse downward by the tail until contact with the handle is broken. The distance the mouse pulls the handle is recorded.

Auditory startle is assessed by placing each mouse into a test chamber (MED-ASR-PRO from Med-Associates) for 30 minutes. During this time auditory stimuli (bursts of white noise with a rapid onset and offset) are delivered at variable intervals. The amplitude of each startle is automatically recorded. A normal mouse will show high amplitude startles at first followed by some habituation over the session.

Visual discrimination is assessed using the Morris Water Task (MWT) apparatus. Mice are released into a pool of water that contains 2 platforms. A striped white and black ball marks one of the platforms and a white ball marks the other. Only the striped ball affords an escape from the water while the white ball is unstable. The number of errors (i.e., making contact with the unstable platform) is recorded. A mouse with normal visual acuity will learn to swim quickly and unerringly to the striped ball to escape the water.



Tailhang

NeuroInvestigations

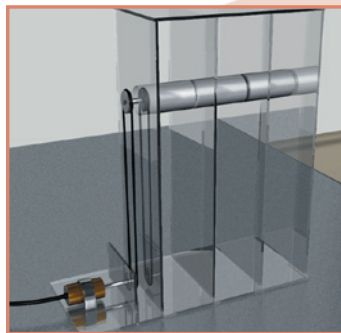
Mouse Phenotyping

Pain Perception

Von Frey Hairs can be used to test pain threshold. Beginning with the finest hair, each hindlimb is poked with sufficient pressure to bend the hair slightly. This is repeated with increasingly firm hairs until an obvious withdrawal response is observed in both hindlimbs. The number of the hair that invokes this response is recorded and considered the pain threshold.

Tail flick provides a measure of the pain response. The mouse's tail is exposed to the surface of water that is heated to 60°C. The latency to flick the tail away from the heated surface is recorded.

Formalin test is a test of pain sensitivity. The mouse is lightly anaesthetized with Isoflurane gas anaesthetic. A subcutaneous injection of 2% paraformaldehyde is delivered to the right hind paw. Time spent licking paw, total number of paw licks and duration of each paw lick is recorded for 50 minutes following recovery from anaesthesia.



Rotarod

Tests of balance and coordination

The rod test assesses balance using a narrow wooden rod that is suspended horizontally between two small platforms 30 cm above the surface of a table. Each animal is held upside-down by the base of the tail and then placed against the wooden rod allowing the animal to grasp the rod only with its hind limbs. The tail is then released. The amount of time it takes the animal to pull itself into a balanced position upon the rod is recorded.

Rotarod is a test of balance and motor coordination. All mice are trained for several days to walk on a rotating drum. During each test session the drum is rotated at 10 rpm in the initial session and then this speed is increased by 5 rpm in each subsequent session to a maximum of 45 rpm in the final session. The amount of time that the mouse remains on the drum per session is recorded as well as the number of times the mouse holds the rod and rotates or loops around with it.

Tests of cognition

The Morris water task is the gold standard for measuring spatial learning and memory in rodents. Each mouse is placed into a round pool of opaque water from different start locations. The mouse is allowed 1 min to swim about the pool and locate a safety platform that is hidden just under the surface of the water. There are no local cues to guide the mouse only distal cues around the pool. The only way to quickly and reliably locate the platform from any start location is to learn and remember the locations of the distal cues and use them to guide swimming to the platform. Time to find the hidden platform, distance swam and route taken are all recorded and analyzed. Normal mice learn quickly to swim to the platform when released from any area within the pool. On the 20th trial a Probe trial is performed. On the Probe trial the platform is removed from the pool and the mouse is allowed to swim for 30 s. The dwell time and distance traveled in each quadrant in the pool are recorded. Normal mice spend most of their swim time and distance within the quadrant where the platform had been located during the previous 19 trials. Finally, we test the mouse's ability to learn about a new location within the pool by moving the platform to the opposite quadrant of the pool. Normal mice learn quickly that the position has changed and begin swimming to the new location after only a couple trials.

Fear (trace) conditioning measures classically conditioned learning and memory involving affect (i.e., fear). In this test each mouse is placed into an apparatus that contains a speaker and a grid floor through which shock can be delivered. Following a period of habituation to the chamber each mouse receives 20 trials during which a tone is sounded for 15 s. Thirty seconds following the offset of the tone a foot shock (.7 mA) is delivered through the floor grid. Twenty-four hours later each mouse is placed into the test apparatus and the same program of 20 tones is run, however, during this session there are no shocks delivered. The amount of time that each mouse spends 'freezing' (absolutely still) is calculated. Normal mice show little freezing during the tone, and significant freezing at the time when the shock is predicted to occur (i.e., 30 s following the tone offset).

NeuroInvestigations

Mouse Phenotyping

Place preference is measured by placing the mouse in an apparatus that is composed of 2 large compartments. They are similar in size, shape, illumination, and such, but they are distinctive in wall patterns. One room has black circles on white walls while the other has plain white walls. A small start box connects the two compartments. The mice are food restricted for 4 hours prior to the test session. Each mouse is placed in the start box and one of the compartments contains a food reward. The mouse is allowed to eat the reward. The following day each mouse is placed into the start box and the time spent in each room is recorded over 5 minutes. Normal mice remember that food was consumed in one particular room and, hence, spend the majority of time in that room.

Recognition memory is tested by placing each mouse into a test chamber that is a small rectangular square made out of black Plexiglas. The chamber contains two small round windows situated on opposing sides of the chamber. Each window is equipped with a shutter and laser beam tracking. The first day the mice are placed into the chamber for 5 min with one window open. The second day they are placed into the chamber for 5 min with both windows open. The number of times the mouse pokes its head out each window is recorded. Normal mice will prefer the newly unshuttered window, to the previously exposed window.

Prepulse inhibition is a test of attentional processes using suppression of startle as the measure. Each mouse is placed in the test chamber (MED-ASR-PRO from Med-Associates). Following a habituation period, 30 startle stimuli (white noise with sharp onset and offset) are delivered at random intervals to measure baseline startle amplitude. Following baseline startle stimuli are randomly delivered with tones interspersed at varying intervals before the startle stimuli. In a mouse with normal attentional abilities the startle response will be dampened to a greater degree with shorter intervals between the tones and the startle stimulus. The longer the interval between the tones and the startle stimulus the greater the startle amplitude.

Tests of activity cycles

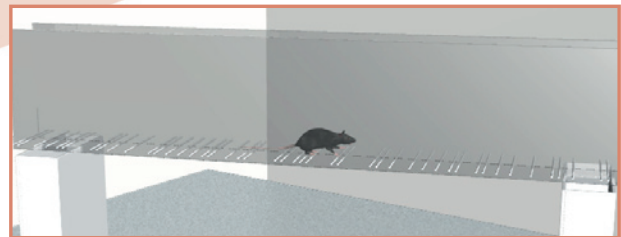
Open Field Activity measures activity level in a novel environment. The mice are individually placed in a clear, plastic box (36 x 36 cm) with surrounding infrared sensors. They are left in this box for 10 minutes in conditions of low noise and dim lighting. Total horizontal distance (in cm) and number of vertical movements (rears) are tracked by

the infrared sensor system. The number of fecal pellets excreted is also counted for the 10 min duration. Normal mice show an initial burst of rearing and movement and then a steady lower rate of exploration.

Circadian Activity measures 24-hour activity cycles. The mice are individually housed in hanging fitted with photocell sensors. The mice are monitored for 24 hours in a 12-hour light, 12-hour dark cycle. Following this, the animals are monitored for several 24-hour sessions in a darkened setting. The number of cage crosses is tracked by computer and reported in 30-minute intervals. Normal mice show an initial burst of exploration activity followed by a relatively low level of activity through the rest of the light cycle. The dark part of the cycle will show regular peaks and valleys of activity throughout. During the test of consecutive dark-only sessions the mice show alternating periods of low and high levels of activity allowing for measurement of the integrity of normal endogenous rhythmicity.

Tests of motor skill

The **Grid Walk** test is a test of motor coordination. The apparatus consists of a bridge made of two tall Plexiglas walls connected by a floor of small, round irregularly spaced bars forming a grid. The bridge is suspended 1 m above the table surface. Each mouse is placed at one end of the bridge and videotaped from the side as they walk across the grid to the opposite end. The number of forelimb and hind limb placement errors as the mouse traverses the bridge scored. An error is counted whenever a limb misses a bar and extends downward through the plane of the bars.

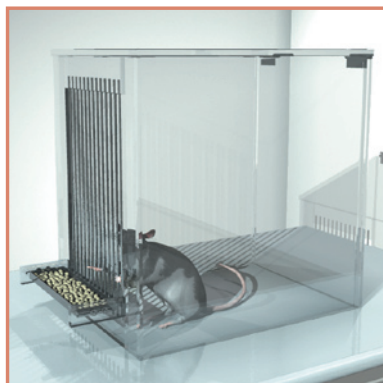


Grid Walk

NeuroInvestigations

Mouse Phenotyping

The **reaching task** measures a mouse's ability to learn a complex motor skill. Each mouse is placed daily into a testing box that has narrow slats along one side. Situated on the outside of the chamber and running along the slats is a food tray. The food tray contains small round sweet pellets. A normal mouse will readily learn to reach its forepaw through the narrow opening of the slat, grab a sweet pellet, and then bring its forearm back into the test chamber to consume the pellet. The total number of attempted reaches and the number of successful reaches (gets pellet into mouth) are recorded.



Reaching Task

Tests of anxiety

The **Elevated plus maze** is a test of anxiety level. The test apparatus consists of 4 arms in the shape of a plus. Two arms are open to the testing room and 2 are enclosed with high walls. A video recorder connected to a monitor is mounted above the maze. Each mouse is placed onto the maze and observed for 4 min. The time spent in the open arms, and total number of open-arm and closed arm entries is recorded. Normal mice experience a high level of anxiety when placed on to the maze and, hence spend a significant amount of time in the enclosed arms where they feel safer.

Fear potentiated startle (FPS) tests anxiety. Each mouse is placed into the testing chamber (MED-ASR-PRO from Med-Associates) for 30-40 min. A total of 30 auditory startle stimuli are delivered at random intervals on 2 consecutive days. The auditory startle stimuli consist of a sharp burst of white noise with a rapid onset and offset. The amplitude of startle is recorded and serves as baseline startle responses. On the 3rd and 4th days each mouse is placed into the test chamber and are given 20 pairings of light (3 s) ending with a shock (.7 mA) delivered through the floor of the test chamber.

On the 5th and final day each mouse is placed into the test chamber and given 30 startle stimuli trials alone intermixed with 30 light plus startle stimuli trials. Startle amplitudes are recorded and analyzed for the startle alone trials and the light plus startle trials. A normal mouse will show an enhanced startle to the light plus startle trials when compared with the startle stimulus amplitudes alone.

Species typical behavior

Social conflict in male mice is a test of male aggressiveness. A novel male mouse (weight and age matched) is introduced into the home cage of each individually housed experimental mouse for a 5 min session. The session is videotaped and scored for the number of bites and pins delivered to the intruder.

Sexual behavior of each individually housed experimental male mouse is analysed by placing an estrous female into the home cage for a 10 min session. Each session is recorded and scored for latency and number of mounts, latency of and number of intromissions, and penis licking.

Nest building is observed and measured by introducing paper ribbons to each cage. The following day the resulting nests are scored. A normal mouse will use the paper ribbons to build a tightly woven nest that has an obvious entrance and ceiling.

For more information

Including price quotes and customized testing batteries
please contact:

Janice Sutherland, Ph.D.

Phone: (403)-394-3978

Fax: (403)-329-2775

Email: j.sutherland@neurodetective.com

Address: NeuroDetective, Inc. - CCBN

4401 University Drive

Lethbridge, Alberta, Canada

T1K 3M4